Magnetically orientable phospholipid bilayers containing small amounts of a bile salt analogue, CHAPSO

Charles R. Sanders II and James H. Prestegard
Department of Chemistry, Yale University, New Haven, Connecticut 06511 USA

ABSTRACT Buffered mixtures of the detergent 3-(cholamidopropyl)dimethylammonio-2-hydroxy-1-propanesulfonate (CHAPSO) and dimyristoylphosphatidylcholine (DMPC) orient in the presence of a strong magnetic field over a wide range of water contents (at least 65–85%) and CHAPSO:DMPC molar ratios (typically 1:10–1:3). ^{31}P NMR studies show that the phospholipid in such mixtures is oriented with its director axis perpendicular to the magnetic field. ^{31}P and ^{2}H NMR results also suggest that the structure and dynamics of the DMPC molecules are similar to that of pure phospholipids existing in the liquid crystalline (L_α) bilayer phase. The ability of 1:5 CHAPSO:DMPC samples to orient is highly tolerant of large changes in temperature, pH, and ionic strength, as well as to the addition of substantial amounts of charged amphiphiles or soluble protein. However, ^{2}H NMR studies of deuterated β-dodecyl melibiose (DD-MB) solubilized in the system indicate the head group conformation and/or dynamics of this glycolipid analogue is dependent upon the CHAPSO concentration. Despite the latter results, the orientational versatility of the system, together with the nondenaturing properties of CHAPSO, makes this system useful in spectroscopic studies of membrane-associated phenomena.

INTRODUCTION

The use of model membrane systems based on the phospholipid bilayer, such as liposomes, unilamellar vesicles, and certain discoidal micelles, has been essential in the development of our understanding of the structure and function of biological membranes. One of the primary techniques utilized in this study of these model systems has been nuclear magnetic resonance (NMR). Whereas both solid-state and solution NMR methods have been employed, the former has been particularly useful (1–6).

For solids NMR lipid assemblies are usually randomly dispersed and tumbling at a rate which is slow on the NMR time scale. These systems yield powder patterns with shapes that can be analyzed in terms of the geometry and motional restriction of the molecule under observation as long as simple rotational reorientation about the bilayer normal can be assumed. While powder patterns have proven useful, information can be difficult to extract when motions are complex and when powder patterns from several different sites are superimposed. Broad powder patterns also tend to result in poor sensitivity. Rather than having intensity concentrated in discrete resonances of a few hertz line width, as in solution NMR, intensity is distributed over the thousands of hertz typically covered by a powder pattern.

A compromise between the extremes of solution and solids NMR involves the use of oriented samples. Spectra from such samples have single high resolutionlike resonances (or multiplets) for each chemically distinct species, with spectral parameters (chemical shift, relaxation rates, and quadrupolar/dipolar coupling constants) dependent upon the geometric (orientational) relationship of a spin interaction tensor to director axes describing sample orientation in the magnetic field. Producing such spectra from powder patterns is the objective of computational procedures such as "de-Pakeing" (7). Unfortunately, de-Pakeing requires several simplifying assumptions and only partially improves the sensitivity problem. The use of physically oriented samples is preferred whenever experimentally possible.

The physical orientation of lipid bilayers for use in NMR studies has usually been accomplished via one of three methods. The first involves coating glass plates with phospholipid dispersions to produce multilamellar assemblies oriented with the bilayer normals orthogonal to the plane of the plates (8–11). The second method relies on the diamagnetic anisotropy and high cooperativity of amphiphiles making up nematic liquid crystals to magnetically induce orientation of the micellar systems when placed in a strong magnetic field (12–14), and the third involves the orientation of liposomes by a magnetic field (15–17).

All of the above methodologies, as currently practiced are subject to a number of technical and biochemical difficulties which have limited their broad application to problems in membrane biochemistry. The preparation of oriented multilayers on glass plates is both difficult and labor-intensive, and results in the sensitive volume of the NMR probe being occupied more by glass than by the sample of interest. Also, titration of such samples to study the effect of pH, various ions, and other surface active

0006-3495/90/08/447/14 $2.00

agents is difficult if not impossible. Whereas the nematic systems are generally much easier to prepare and can lead to much higher NMR sensitivity, their biological application is somewhat questionable because the amphiphiles most frequently used, such as cesium perfluorooctanoate, potassium laurate, and hexadecyltrimethylammonium bromide, are not typical components of biological membranes and, in many cases, are protein denaturants. In the few instances where orientation of liposomes has been achieved, we find homogeneity and the multilamellar nature of the bilayers present in the preparations to be less than ideal. In particular, inner layers of bilayers are not easily accessible to added ions and other polar molecules. To a certain extent, a similar problem exists with glass plate methods and methods based on nematic liquid crystal behavior in that they usually require the water content to be rather low, making studies which require a substantial volume of water above the surfaces (such as in studying the interaction of soluble proteins with membrane-bound receptors) difficult.

Previous work reported from our laboratory (18) indicated that a magnetically orientable nematic system more closely approaching a lipid bilayer could be reproducibly generated from DMPC-bile salt mixtures. However, sample preparation was somewhat time consuming and the water content of samples investigated was still quite low. Bile salts can also cause protein denaturation (19). We now report the development of a related system which overcomes these difficulties. Our results indicate that over a considerable range of water content, easily prepared mixtures of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 3-(cholamidopropyl)dimethylammonio-2-hydroxy-1-propanesulfonate (CHAPSO), a non-denaturing zwitterionic bile salt derivative (20–21), form magnetically orientable bilayer assemblies at 40°C. Further investigation of 1:5 CHAPSO:DMPC mixtures indicated orientation was maintained over a wide range of temperature, pH, ionic strength, and in the presence of substantial amounts of positively and negatively charged amphiphiles and soluble protein.

MATERIAL AND METHODS

Materials

DMPC, 1,2-dilauroyl-*sn*-glycero-3-phosphoric acid (disodium salt) (DLPA), and CHAPSO were purchased from Sigma Chemical Co. (St. Louis, MO), β-dodecyl maltoside (DD-Malt) from Calbiochem-Behring Corp. (La Jolla, CA), Raney Nickel, deuterium depleted water, hexadecyl trimethylammonium bromide (HDTMA), and decylamine (DA) from Aldrich Chemical Co. (Milwaukee, WI), heptadecylamine (HDA) from Pfaltz and Bauer (Stamford, CT), 1,2-dimyristoyl-*sn*-3-glycero-phosphoethanolamine and acyl perdeuterated DMPC from Avanti Polar Lipids, Inc. (Birmingham, AL).

Concanavalin A (Con A), used to test the effect of added protein upon the orientational properties of the CHAPSO-DMPC system, was purchased from Calbiochem-Behring Corp. and converted to the Co^{2+} substituted form following published procedures (22). Myoglobin and lysozyme used in ^1H NMR studies of protein denaturation were purchased from Sigma Chemical Co. and were prepared for final dissolution in a buffered D_2O solution by first dissolving the protein (50 mg/ml) in a 10 mM *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid) (Hepes), pH 7.2 D_2O solution followed by lyophilization to exchange labile protons.

β-Dodecyl melibiose (DD-MB) was synthesized (Sanders, C. R., and J. H. Prestegard, unpublished results) and partially deuterated at various positions on its pyranosyl rings by refluxing (for up to 1 h) 100 mg of the glycoside in 3.5 ml of D_2O and 7 ml of dimethyoxy ethane in the presence of 1–2 ml of well-rinsed and D_2O-exchanged Raney nickel (23) (it should be noted that dry Raney nickel can ignite organic materials). This treatment was followed by purification by flash chromatography of the filtered and dried product on a column (1 cm × 10 in) of silica gel eluted with 2:1 (vol/vol) chloroform/methanol. The product was typically isolated at a 50% yield and judged to be extensively deuterated at its disaccharide head group and >90% pure using ^1H NMR at 500 MHz. The same method was used to deuterate β-dodecyl maltoside (DD-malt).

Preparation of the CHAPSO-DMPC samples

1-ml samples were prepared in 7 mm NMR tubes by weighing DMPC and CHAPSO at the appropriate molar ratios into the tube, followed by dilution to 1 ml with deuterium-depleted buffer (usually 0.1 M Hepes, pH 7.8). The NMR tube was then sealed with Teflon tape, capped, and resealed so that it could be mixed using a hand-cranked centrifuge without leaking. Thorough mixing was accomplished by a combination of heating (up to 100°C), cooling (on ice), vortexing, and hand centrifuging. DD-MB or DD-malt, when included, was dissolved in buffer or in water and added to the CHAPSO and DMPC in the NMR tube. Titration of DMPC samples was carried out by adding either buffered CHAPSO or buffer and solid CHAPSO directly into the sample in the NMR tube. This method was also used when CHAPSO-DMPC samples were titrated with DLPA, HDTMA, DA/HDA, KCl, and protein. CO^{2+}Con A, lysozyme, and myoglobin were added to CHAPSO-DMPC-buffer samples as buffered stock solutions, 3 mM in monomeric protein.

NMR methods

All experiments were run unlocked and without sample spinning on a model AM-500 spectrometer (Bruker Instruments, Inc., Billerica, MA) equipped with a variable temperature control unit and a 10-mm high-resolution broadbanded probe. The ^2H (77 MHz) and ^{31}P (202 MHz) 90° pulse lengths were ~23 ms. ^{31}P spectra were acquired using either composite pulse (WALTZ)^1H decoupling (decoupler power = 10–15 W, ^1H 90° pulse = 40 μs) and 35° ^{31}P pulses, or without decoupling using ~ 80° pulses. The ^{31}P spectra presented in this paper were acquired by taking 2,048 time domain data points over spectral widths of 25,000–36,000 with pulse repetition times of 2–3 s and are referenced to external 50% phosphoric acid. ^2H NMR free induction decays (FIDs) were acquired using simple 80° pulses or the solids echo sequence (24) over sweep widths of 50,000–120,000 Hz with 2,048–8,096 data points being taken and pulse repetition times of 0.08–0.2 s. Samples were usually cooled on ice before being warmed to the desired temperature in

the magnet of the spectrometer and were typically allowed to equilibrate for 15 min before acquisition.

RESULTS

Orientational properties of the CHAPSO-DMPC-buffer system

DMPC suspensions at 35 and 15% (wt/vol) in buffer at 40°C were titrated with CHAPSO and buffer such that the percentage of total lipid (DMPC + CHAPSO) was not varied. The spectrum for pure 35% DMPC is shown at the bottom left of Fig. 1. The spectrum is dominated by the chemical shift anisotropy and yields the asymmetric powder pattern characteristic of axially rotating phospholipid in L_α bilayers. The maximum of the pattern at -14 ppm represents the fraction of the DMPC population whose axis of rotation (director) is oriented at a 90° angle with respect to the external magnetic field, and the minimum at ~30 ppm represents the faction whose axis is at 0° (4).

The titration of 35% DMPC with CHAPSO is accom-

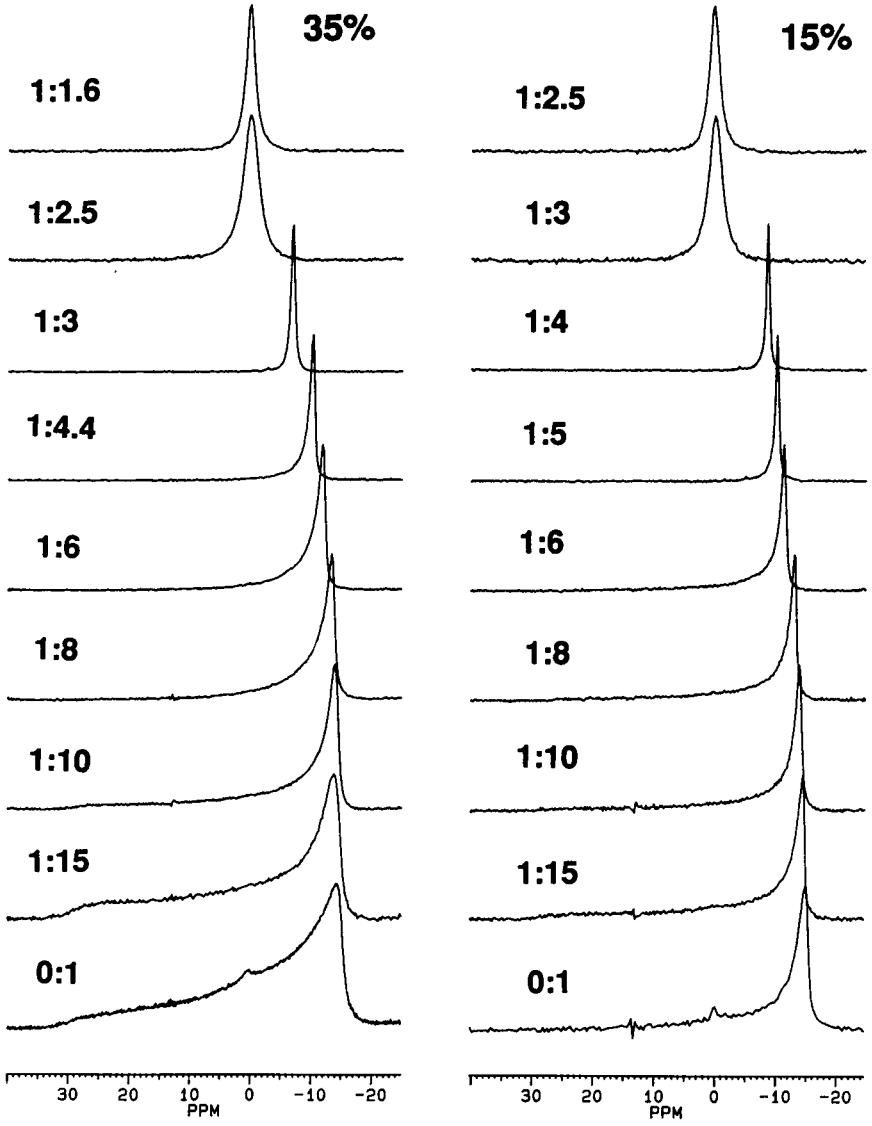

FIGURE 1 ^{31}P (^1H-decoupled) spectra at 35 and 15% (wt/vol) total lipid (CHAPSO + DMPC) in 100 mM Hepes buffer, pH 7.8 at 40°C at various CHAPSO:DMPC molar ratios. Spectra were processed using 20 Hz of exponential line broadening and typically represent 150 scans. Acquisition parameters are described in Materials and Methods.

panied by two major changes in the ^{31}P spectrum. First, there is a gradual disappearance of the powder pattern as a single resonance becomes increasingly dominant. Secondly, the major component gradually shifts from its initial position at -14 ppm to a final position at 1 ppm. The convergence upon 1 ppm is rather abrupt and is also accompanied by a sudden increase in linewidth. The reduction of the powderlike nature of the spectrum is accounted for by the DMPC assemblies present in the sample becoming preferentially oriented by the magnetic field. This interpretation is supported by the fact that immediately after placing prewarmed samples in the magnet, the ^{31}P resonance is difficult to detect because it is broad and asymmetric. After a short period of time (<5 min) sensitivity increases and the spectra reveal considerable orientation. Higher CHAPSO:DMPC ratios and lower overall percentage of lipid appears to produce somewhat more rapid orientation.

The downfield shift of the oriented component stopping at 1 ppm is consistent with several possibilities. The position is that expected for DMPC which can execute isotropic motion. It is also that expected for motional averaging at the "magic angle" (54.7°), and it is that expected for a phosphorus chemical shift tensor which has undergone a change in local orientation such that its principal component is now 54.7° relative to the axis of motional averaging (25). The latter two possibilities are ruled out by the fact that the changes in the ^{31}P chemical shift as a function of CHAPSO addition is mirrored by reductions in the quadrupolar splittings of deuterated DMPC (Figs. 2 and 3) and DMPC-associated glycolipids (Figs. 5 and 6). These changes cannot be accounted for by a change in headgroup conformation. The most probable explanation for the shift, and the one which is in complete accord with the current understanding of bile salt-phosphatidylcholine (PC) interactions (see Discussion) and the ^{2}H NMR data is that while the axis of motional averaging maintains an average orientation of 90° with respect to the magnetic field, the lipid assemblies are becoming increasingly mobile until the isotropic limit is reached. The broadening at the region of abrupt shift may suggest that both oriented and isotropic components are in phase equilibrium with rapid or intermediate exchange of molecules between domains.

The titration of 15% DMPC was also followed by ^{31}P NMR and was observed (Fig. 1) to exhibit orientational properties similar to the 35% case with two major exceptions. First, in the absence of any added CHAPSO, 15% dispersions showed significant magnetically-induced orientation at 40°C. This is not surprising because other investigators have reported the magnetically-induced orientation of aqueous dispersions of some types of lipid bilayers in magnetic fields (15–17). The lower concentration very likely improves the kinetics of reorientation. Second, the 15% dispersion became isotropic at a slightly lower CHAPSO:DMPC ratio relative to the 35% case. These concentration-dependent trends were confirmed with 5 and 25% samples.

The resonance asymmetry observed in many of the "substantially-oriented" spectra of Fig. 1 can be accounted for by a certain fraction of the DMPC present being completely disoriented so that a powder pattern is superimposed upon the oriented component and/or by distribution of a fraction of the lipid over a range of orientations close to, but not at the ideal orientation of 90° (8).

^{2}H NMR can be used to extend characterization of the oriented mixtures into the hydrocarbon matrix of the bilayer. Fig. 2 shows the ^{2}H NMR powder pattern for acyl perdeuterated DMPC in 35% DMPC (*A*) along with the spectra from 1:5 CHAPSO:DMPC at various levels of buffer (*B–E*). The 1:5 samples all give rise to a series of doublets, one for each site in the perdeuterated molecules. The increase in resolution due to orientation is dramatic.^{31}P spectra are shown for comparison.

Fig. 3 shows first the increasing resolution as the samples orient and then the reduction and eventual loss of splittings from d_{54}-DMPC as 15% DMPC is titrated with CHAPSO. This is consistent with the increasing orientational homogeneity and mobility of the lipid assemblies at higher levels of CHAPSO.

Affect of environmental factors on orientational properties

For practical reasons, we attempted no systematic examination of the environmental dependencies of this system over a wide range of CHAPSO:DMPC ratios. However, a number of observations regarding the orientational tolerance of 1:5 samples to variation in sample composition and temperature are summarized below. Given the similarity of the 1:5 spectra shown in Figs. 2 and 3 over a 10-fold range of total lipid concentrations along with the gradual changes in Fig. 1 as CHAPSO is varied, it seems probable that the following specific observations are applicable to a much wider range of compositions.

Temperature

Fig. 4 shows the spectra from a 35% 1:5 sample over a range of temperatures. Orientation is maintained from at least 30–65°C. It is interesting to note that the 24.5° ^{31}P spectrum appears to exhibit orientation while ^{2}H NMR does not. This may be an artifact of incomplete motional

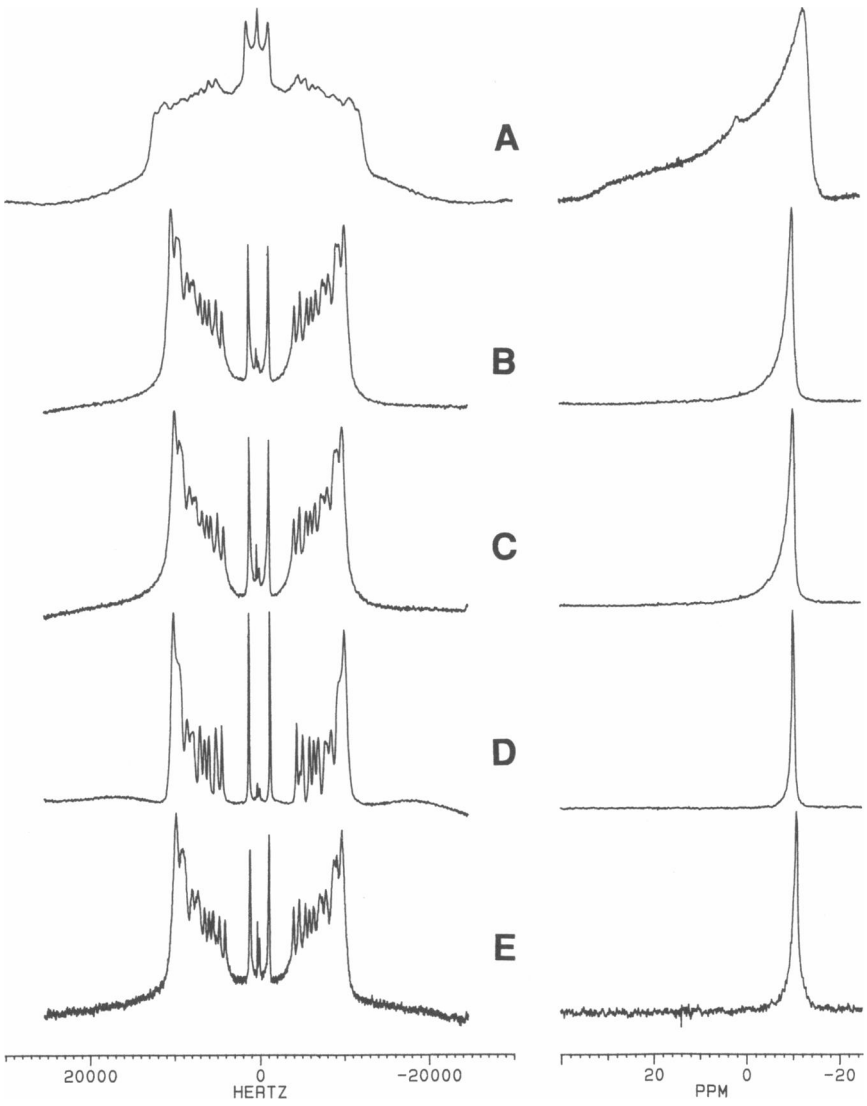

FIGURE 2 ^{2}H NMR spectra of acyl perdeuterated DMPC at 40°C in 100 mM Hepes deuterium-depleted buffer at pH 7.8 and varying amphiphile compositions: (*A*) 35% (wt/vol) DMPC; (*B*) 50% total lipid (DMPC + CHAPSO), 1:5 (mol:mol) CHAPSO:DMPC; (*C*) 35%, 1:5 CHAPSO:DMPC; (*D*) same as *C* except sample was heated to 65° and cooled back to 40° in the magnet of the spectrometer before acquisition; (*E*) 5%, 1:5 CHAPSO:DMPC. FIDs were acquired using the solids echo sequence as described in Methods and were processed with 75 Hz (spectra *A*) or 20 Hz (*B–E*) of exponential linebroadening. Spectrum *A* was acquired using composite 90° pulses (37). Samples contained 4–20 mg of d_{54}-DMPC in excess unlabeled lipid.

averaging in the deuterium case. It is easier to average the ^{31}P chemical shift anisotropy of order 10 kHz that the deuterium quadrupole coupling of order 100 kHz. Below 24.5° spectra characteristic of orientation are lost but it is unclear whether this is due to actual disordering of the lipid assemblies or whether it simply reflects the loss of axial motional averaging for DMPC molecules within the assemblies. At 65° isotropic and oriented phase resonances are observed in both ^{31}P and ^{2}H spectra. This supports possible coexistence of isotropic and oriented phases, suggested previously on the basis of linebroadening at high CHAPSO:DMPC ratios (Fig. 1).

Ionic strength, pH, and added protein

The lack of significant changes in either the ^{31}P (^{1}H coupled) or the ^{2}H NMR spectra from solubilized DD-MB or β-dodecyl maltoside (data not shown) indicates 15% 1:5:0.5 CHAPSO:DMPC:DD-MB (or maltoside) samples remain substantially oriented at 40° regardless of

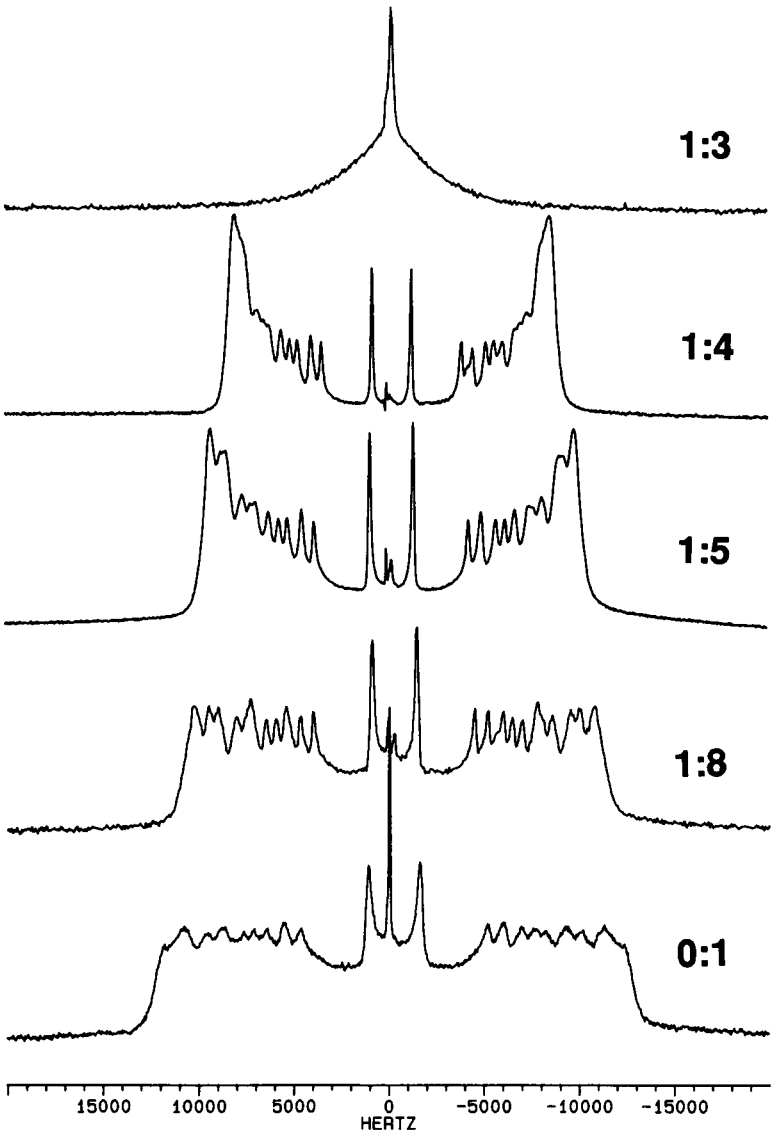

FIGURE 3 Dependence of the solids echo ^2H NMR spectra from d_{54}-DMPC as the level of CHAPSO is varied. Samples contained 3–10 mg of deuterated DMPC in excess unlabeled DMPC and were 15% (wt/vol) in total lipid (DMPC + CHAPSO) in 100 mM Na^+Hepes deuterium-depleted buffer. Spectra are labeled with the CHAPSO:DMPC molar ratio and were produced following exponential multiplication of the FIDs with 20–30 Hz of linebroadening.

whether they were buffered with 0.1 M pH 7.8 Hepes, with 75 mM sodium acetate, 10 mM $CaCl_2$, 2 mM $CoCl_2$, pH 5.2, or unbuffered with KCl varying in concentration from 0 to 0.5 M. The addition of Co^{2+}Con A to the acetate buffered sample (to 37 mg/ml, diluting the lipid to 12%) also resulted in no change.

Added amphiphiles and protein

Other amphiphiles can be incorporated into these orientable systems. For example, Fig. 5 shows the ^2H NMR spectra arising from DD-MB which is deuterated at several sites on its sugar head group and which is solubilized in 25% CHAPSO-DMPC samples. This glycolipid analogue is clearly associated with the CHAPSO-DMPC assemblies and substantially oriented as shown by the doublets arising from each side. Fig. 6 shows similar spectra from DD-malt solubilized in 15% CHAPSO-DMPC samples.

DLPA, alkyl ammonium salts, and HDTMA, when added to 15% 1:5:0.5 CHAPSO:DMPC:DD-MB samples

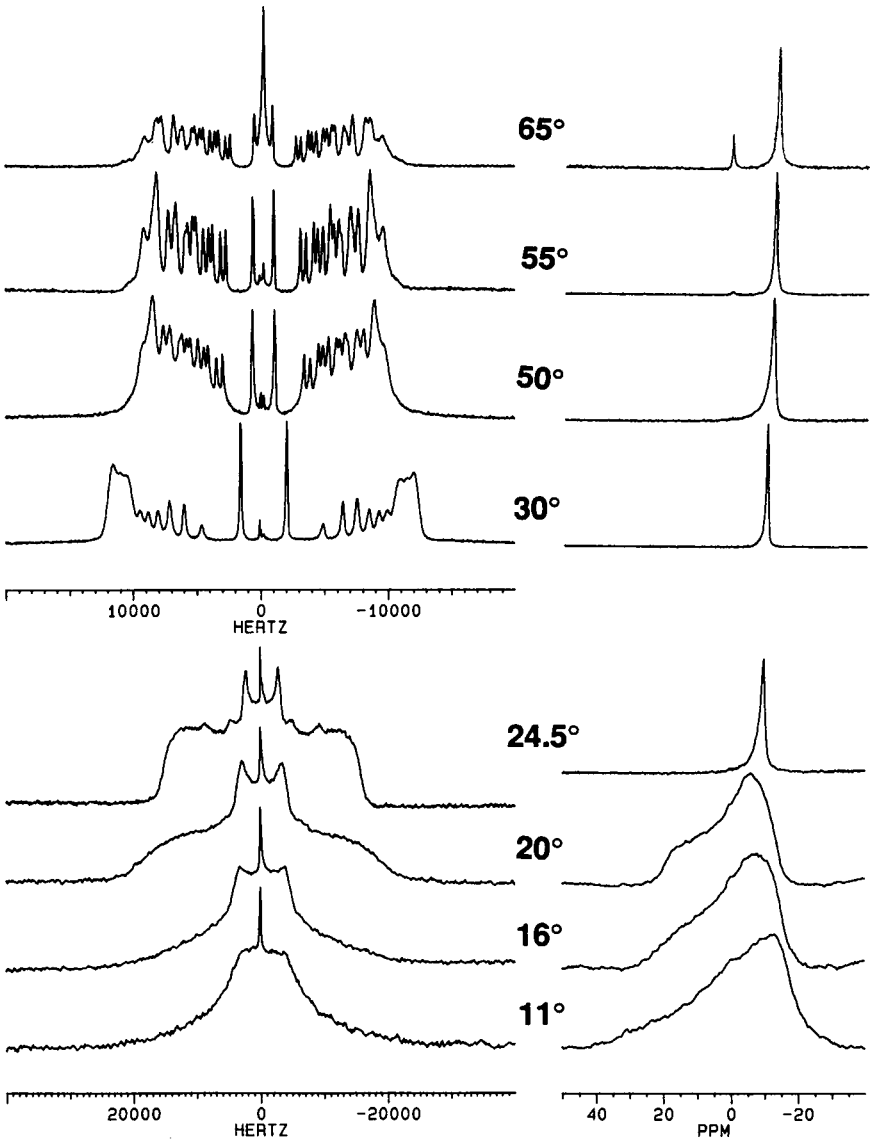

FIGURE 4 Temperature dependence of spectra from 35% total lipid, 1:5 CHAPSO:DMPC. ^2H NMR spectra (left column) were produced from FIDs acquired using the solids echo sequence processed with either 100–200 Hz (lower spectra) or 20 Hz (upper) of exponential linebroadening. ^{31}P (^1H-decoupled) spectra (right column) were acquired as described in Methods and processed in the same manner as the ^2H spectra. Samples contained 20 mg of acyl perdeuterated DMPC in excess unlabeled lipid and were buffered with 100 mM Na^+Hepes.

to amphiphile:DMPC ratios of up to 1:2 did not result in disorientation of the system as judged by the ^2H NMR signal from solubilized β-DD-MB and ^{31}P NMR signal from the DMPC. Above this ratio the system tended to break down. At high levels of DLPA or alkylammonium salts the samples became visibly inhomogeneous. Oriented and (apparently) isotropic components were observed to be simultaneously present by ^{31}P NMR. Large amounts of HDTMA resulted in the visual clarification of the CHAPSO-DMPC and a shift of the ^{31}P resonance towards its isotropic position.

Effects of CHAPSO on soluble protein structure

We examined the ^1H NMR spectra of myoglobin and lysozyme in both a simple aqueous solution (100 mM Hepes, pH 7.4 in D_2O) and in a 12% 1:4.5 CHAPSO-

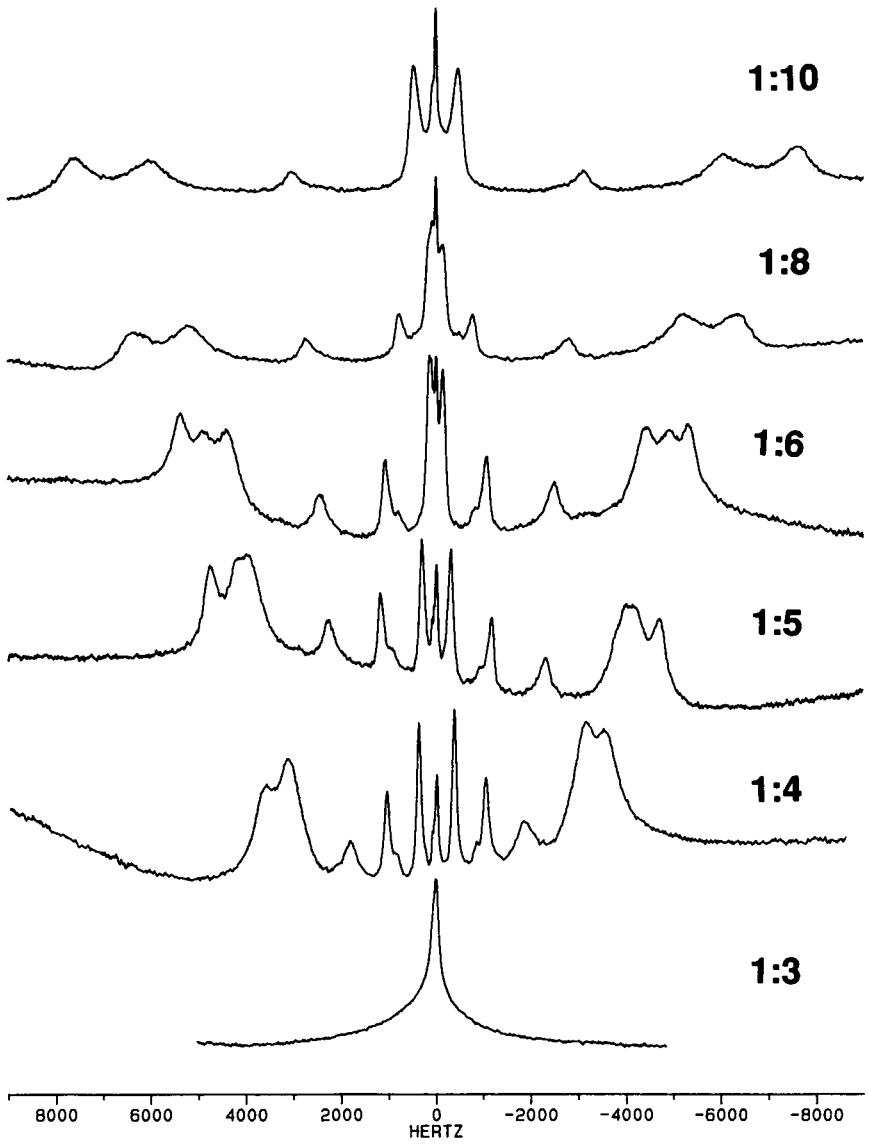

FIGURE 5 Dependence of the ^2H NMR (single pulse sequence) spectra of partially deuterated β-dodecyl melibioside on the CHAPSO:DMPC molar ratio. Samples contained 10 mg of partially deuterated DD-MB in 25% (wt/vol) total lipid (CHAPSO + DMPC) in with 100 mM deuterium-depleted Na^+Hepes deuterium-depleted buffer, pH 7.8, 40°C. FIDs were processed with 20 Hz of exponential linebroadening.

DMPC D_2O solution (buffered at pH 7.4 with 100 mM Hepes) to exclude the possibility that the system induces protein denaturation. While the aliphatic region of the spectra from the liquid crystalline samples is partially obscured by the resonances arising from the CHAPSO and DMPC, only protein peaks are present in the aromatic regions of the NMR spectra. The aromatic region of the ^1H NMR spectra are virtually identical (data not shown) in the absence and presence of the CHAPSO-DMPC mixture for both proteins. This indicates that the system does not perturb the structures of these proteins and that there is sufficient bulk water in the liquid crystalline samples to allow the proteins to diffuse isotropically in solution despite the fact that the lipid assemblies are oriented.

DISCUSSION

Morphology of the oriented CHAPSO-DMPC system

CHAPSO is a cholic acid derivative in which the carboxylic acid has been amidated with a zwitterionic aminosulfonic acid moiety. Thus, it retains the same hydrophobic/

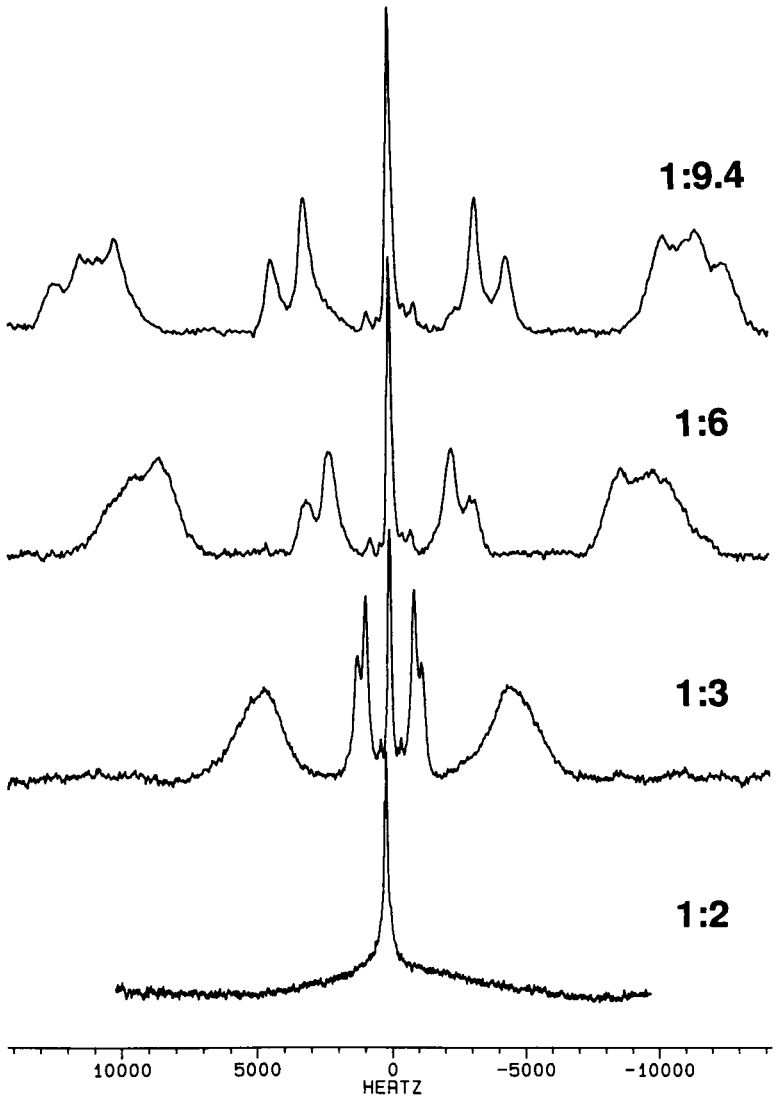

FIGURE 6 Dependence of the ^2H NMR (single pulse sequence) spectra of partially deuterated β-dodecyl maltoside on the CHAPSO-DMPC molar ratio. Samples contained 10 mg of DD-malt in 35% (wt/vol) total lipid (DMPC + CHAPSO) buffered with 100 mM Na^+Hepes, pH 7.8 at 40°C. FIDs were processed with 20–75 Hz exponential line broadening and interactive polynomial or cubic spline baseline correction.

hydrophilic topology as the rest of the bile salt series except that it is formally neutral. In addition to the hydrophilic "headgroup" extending from the end of the cholane ring system, it has three hydroxyl groups located on the same side of the rigid multicyclic hydrocarbon making that side of the molecule polar. This topology allows the bile salts to stabilize the edges of lipid bilayers. Studies of aqueous bile salt-PC mixtures have led to some understanding of the compositional dependency of the morphology of bile salt-PC aggregates (26–28). Provided the PC concentration is above ~50 mM, as in all of the samples used in this study, the bile salt:PC dependence of morphology is fairly simple: as PC bilayers are titrated with bile salts the extended lamellae are broken up into disk-shaped micelles in which the bile salt stabilizes the edge of PC bilayers. In addition, there may also be some self-association within the interior of the bilayer disks as well. As the ratio of bile salt to PC is raised to ~1:1 a second morphological transformation to spherical micelles occurs.

If indeed the CHAPSO-DMPC system can be described by the above model then it should conform experimentally to predictions which arise from this model. As the milky aqueous dispersions of DMPC are titrated with CHAPSO, the formation of small mixed micelles should be accompanied by the clarification of the sample.

Also, as the large lamellae are broken down into smaller units by CHAPSO the lipid assemblies should become increasingly mobile.

At 40°C 35% lipid solutions are observed to begin to clarify upon addition of CHAPSO to a CHAPSO-DMPC ratio of ~1:7. For 15 and 25% solutions clarification was clearly underway at 1:5 CHAPSO:DMPC, while the 5% lipid solution did not significantly clarify until a ratio of ~ 1:4. In all cases the solutions became totally clear by the point at which the samples became isotropic (~1:2.5 CHAPSO:DMPC). This is as expected for the formation of micellar solutions. The fact that higher molar ratios of CHAPSO were required to bring about clarification at lower levels of total lipid may simply reflect the higher percentage of soluble (monomeric) CHAPSO present at such concentrations. The critical micelle concentration (CMC) of CHAPSO is fairly high (4 mM at an ionic strength of 0.15 [29]), and although the membrane partition coefficient formally governs the distribution of CHAPSO between micellar and isotropic phases, low partition coefficients seem to correlate with high CMCs (30). Thus, a high CMC would suggest significant percentages of CHAPSO to be monomeric at lower lipid concentrations.

The mobility of the lipid assemblies is observed to become higher as the CHAPSO:DMPC ratio is raised. For lipids oriented with the director axis orthogonal to the magnetic field the observed ^{2}H NMR quadrupolar splitting ($\Delta\nu$) at a given site can be described by the following equation:

$$\Delta\nu = S_{\text{micelle}} \cdot S_{\text{mol}} \cdot 3/4 \cdot \frac{e^2qQ}{h} \cdot \left(\frac{3\cos^2\theta - 1}{2}\right), \quad (1)$$

where θ is the angle between the C-D bond vector and the molecular director axis (i.e., the bilayer normal), e^2qQ/h is the quadrupolar coupling constant (assumed to be fixed at ~170 kHz for C-D bonds) and $S_{\text{micelle}}/S_{\text{mol}}$ are order parameters. S_{micelle} represents order of the micelle symmetry axis with respect to the liquid crystal order axis and S_{mol} represents order of the individual lipid molecules, or segments of molecules, with respect to the micelle symmetry axis. The order parameters have a maximum value of 1 for perfect ordering and a value of 0 for isotropic solutions. Assuming that the lipid maintains a basic bilayer morphology throughout the course of titrations of DMPC by CHAPSO, both S_{mol} and θ, for a given site, should vary little during the titrations. On the other hand, the value of S_{micelle}, which is the same for each site, should decrease as the bilayer units become smaller, more nearly spherical, and therefore more independent in their motional reorientation. This has been confirmed both by direct observation of acyl perdeuterated DMPC and by observation of deuterated DD-MB associated with the DMPC-CHAPSO assemblies. Fig. 3 shows the ^{2}H NMR spectra of acyl perdeuterated DMPC in 15% lipid solutions at various CHAPSO:DMPC ratios. A scaling down of all of the resolvable doublets by 4–10% in going from 1:8 to 1:5 and 8–15% in going from 1:5 to 1:4 and the eventual convergence to an isotropic solution bears witness to the fairly uniform decrease in S_{micelle} which results from the increased breakdown of lamellae and formation of smaller, more mobile, mixed micelles at the higher levels of CHAPSO. Fig. 6 shows the spectrum of DD-malt partially deuterated on its glycosyl headgroup in 35% CHAPSO-DMPC solutions as the amount of CHAPSO is increased. The splittings decrease as CHAPSO is added in a manner similar to that shown by perdeuterated DMPC and eventually collapse to superimposed isotropic singlets. Again, this is fully consistent with the formation of increasingly isotropic micelles.

The actual change in values of the splittings as one moves down the lipid chain are quite characteristic of the conformation and mobility of lipids in typical bilayers. The dynamics of the acyl chains of DMPC and DPPC in L_α bilayers have been the subject of a number of ^{2}H NMR studies (2, 5, 31–33) and it has been established that except for the 2 position on the *sn*-2 chain, the methylene groups near the headgroup are very similar in their average orientation and mobility until a point about half way down the chain is reached, at which point the mobility of the methylene groups begins to increase significantly. Our spectra (Figs. 2–4) are fully consistent with these previous results. The broad doublets with the largest splittings result from the superposition of resonances from the relatively immobile methylene deuterons near the ester linkage. The series of less intense doublets with decreasing coupling constants result from the stepwise decreases in S_{mol} occurring along the terminal portion of the acyl chains. The mobile terminal methyl groups give rise to the relatively intense doublet having the smallest of the observed coupling constants. In fact, our spectra are very similar to de-Paked spectra obtained from powder patterns taken of acyl perdeuterated DMPC and DPPC in L_α bilayers and multilayers, except that all of the doublets in our spectrum have coupling constants which are scaled down (by ~ 30% for the 1:5 case) relative to those from powder patterns, primarily due to the decreased S_{micelle} of the CHAPSO-DMPC system. This provides very strong evidence that the DMPC in the CHAPSO-DMPC system at 40°C (lamellae and or mixed micelles) not only possesses a bilayer morphology but also exists in an L_α-like dynamic state: approximately that of natural biological membranes under physiological conditions.

The ^{31}P titrations (Fig. 1) are also consistent with increasing mobility as the CHAPSO:DMPC ratio is raised. For lipids executing rapid axial rotation about a director axis perpendicular to the magnetic field the observed ^{31}P chemical shift can be described in terms of a "reduced" shift ($\Delta\sigma$) which is the difference (in parts per million) between the observed chemical shift and its isotropic value (4):

$$\Delta\sigma = S_p \cdot S_{micelle} \cdot 1/6 \cdot (\sigma_0 - \sigma_{90}), \quad (2)$$

where σ_0 and σ_{90} are the chemical shifts for the lipid in 0 and 90° orientations with respect to the magnetic field, $S_{micelle}$ maintains the same meanings as in Eq. 1, and S_P is a measure of the order and orientation of the phosphate group relative to the micellar director axis. The observed shift of the ^{31}P resonance from its initial 90° position to its isotropic value as DMPC solutions are titrated with CHAPSO is, again, fully consistent with a decreasing $S_{micelle}$ and a fixed S_P.

Pure DMPC undergoes a main transition to the liquid crystalline phase at ~ 24°. The temperature dependency of spectra from 35% 1:5 CHAPSO:DMPC (Figs. 2 and 4) suggests that, at least for this particular composition, the dynamics of the DMPC present resembles that of pure DMPC in the same temperature range. From 30 to 65° there seems to be no change in the basic morphology or phase of the system but the acyl chains become substantially more mobile as the temperature is raised in a manner similar to that previously observed by Oldfield et al. (34) for pure, L_α acyl deuterated DMPC over that same temperature range. From 30 to 11°, both ^2H and ^{31}P spectra change in a manner approximating powder spectra previously recorded (5, 34, 35) for pure DMPC (or DPPC) below the main transition. Thus, while it is perhaps misleading to describe the CHAPSO-DMPC system in terms of thermotropic phases, the DMPC present in this sample exhibits temperature-dependent dynamics similar to that of pure DMPC. This is in accord with calorimetric studies carried out on bile salt-DPPC mixtures, which indicated that even at a bile salt:PC ratio of 1:1.5, much higher than in most of the samples of the present study, the characteristic main transition still occurred and at a temperature fairly near to that of pure DPPC (36).

Pure 15% DMPC suspended in buffer was often observed to precipitate out of solution as expected for multilayers. However, 15% solutions of 1:2–1:10 CHAPSO:DMPC remained well dispersed in solution, suggesting that the multilayered nature of the bilayer units is substantially reduced and that this system may prove useful in studies of the interaction of molecules in the bulk aqueous phase with the surface of the bilayers.

Effect of the CHAPSO:DMPC ratio on the interfacial headgroup structure

Whereas DMPC appears to maintain a bilayerlike configuration that seems fairly insensitive to the level of CHAPSO present, it is also important to evaluate the degree to which the presence of CHAPSO may affect other bilayer constituents. This question has been investigated for DD-malt and DD-MB. If CHAPSO does not play a significant role in defining the nature of the bilayer interface, then the only changes in quadrupolar splittings should be uniform reductions in splittings due to a decrease in $S_{micelle}$. If, however, a change in headgroup orientation or local dynamics occurred due to perturbation by CHAPSO, then site-specific changes in θ or S_{mol} (see Eq. 1) would result in the doublets scaling down by different amounts. DD-Malt possesses a headgroup in which glucose is α-1-4 linked to the alkyl glucoside and thus possesses two torsional degrees of freedom at the sugar–sugar glycosidic linkage in addition to the degrees of freedom associated with the alkyl chain. Fig. 6 shows that the doublets from deuterated β-DD-malt tend to scale down rather similarly from point to point, indicating that $S_{micelle}$ is decreasing accompanied by relatively small changes in S_{mol} as CHAPSO is added.

DD-MB provides a contrasting case. It is a glycolipid analogue in which a galactose is linked α-1-6 to an alkyl glucoside and thus possesses three torsional degrees of freedom at the sugar–sugar linkage in addition to the degrees of freedom associated with the alkyl chain. The spectra from this titration are shown in Fig. 6. The doublets associated with the headgroup of β-dodecyl melibioside do not follow the simple $S_{micelle}$ scaling. Whereas preliminary quantitative treatment of this data (Sanders, C. R., and J. H. Prestegard, unpublished results) suggests that the actual structural perturbations evidenced by these differential shifts may be fairly minor, this does indicate that one must remain concerned about CHAPSO effects in future studies utilizing this system.

Mechanism of bilayer orientation

The orientation of bilayer lamellae by a magnetic field results only when the anisotropy in magnetic susceptibility for a given assembly is sufficient to make the difference in energy of interaction for various orientations large compared to kT: $B_0^2\Delta\chi > kT$, where B_0 is the field strength and $\Delta\chi$ is the anisotropy in susceptibility of the assembly. For bilayerlike systems this condition can be fulfilled either by making individual units very large, as in the case of oriented multilayers or by making particles behave cooperatively, as in magnetic ordering of interacting micelles in liquid crystals.

At concentrations of CHAPSO where samples approach optical clarity (below a CHAPSO:DMPC ratio of 1:5) orientation is probably due to a high level of cooperativity because individual micelles should be too small to orient on their own. At lower CHAPSO:DMPC ratios the story could be different. However, the ease with which the system remains dispersed suggests it is not composed of large multilamellar particles but of dispersed bilayer fragments. The susceptibility of such individual fragments is likely to be too low to result in individual orientation. Some level of cooperativity must still exist, even at low total concentration, perhaps because large individual sheets cannot tumble independently even when quite disperse. In a uniform 5% lipid dispersion, simple geometric calculations suggest that a single discoidal sheet of diameter greater than ~ 600 Å would not be able to tumble independently of its neighbors.

Pure lipid multilayers can be sufficiently large to orient independently (15–17). The sporadic orientation behavior, particularly at higher lipid concentrations, can be accounted for by the bilayer sheets being kinetically trapped. Constraining steric interactions with other disoriented bilayers can be such that it is impossible to reach a thermodynamically stable configuration in any reasonable amount of time. This explains the residual disorientation evidenced by many of the ^{31}P spectra of Fig. 1. Heating one of these partially disoriented samples (Fig. 2) to 65°C in the magnet to help overcome kinetic barriers followed by cooling the sample back to 40° resulted in an increase in orientational homogeneity. This kinetic/steric effect also probably explains why it is easier to orient pure DMPC dispersions at lower concentrations.

Relationship of the CHAPSO-DMPC system to previously examined lyotropic nematic liquid crystals

One aspect of the DMPC-CHAPSO system which contrasts previous work (12–14, 18) is that orientation is observed even at very high water contents. It is possible that the apparent contrast is as much a result of the method used to monitor orientation as a property of the systems themselves. In this study, orientation has been evaluated based upon observation of the NMR properties of molecules tightly associated with the bilayers. However, past studies of nematics have sometimes utilized the NMR signals from solvent components (^2H NMR of HDO or ^{23}Na NMR of Na^+) to judge whether or not the micellar system is oriented. In CHAPSO-DMPC samples we also fail to observe splitting of an HDO resonance at 25% and lower total lipid concentrations where lipid components are observed to be highly oriented. This is readily accounted for by rapid exchange of HDO between the increasingly dominant bulk water and oriented surface-associated water molecules, which results in the oriented fraction becoming too small to induce an observable splitting.

The use of signals from a solvent component may also be associated with errors in proper characterization of the orientation of the principle micelle axis (i.e., the bilayer normal) with respect to the magnetic field. In contrast to the observations made here, our previous work on a bile salt:DMPC system suggested the possibility that bilayer disks orient with the bilayer normal parallel to the magnetic field (18). This suggestion was based on sample rotation studies in which an HDO resonance was observed. If a disk is oriented with its normal parallel to the field, ^2H splitting for molecules associated with the bilayer will exhibit a maximum value. Immediately after rotation of the sample by 90° (before the assemblies have had a chance to reorient) all molecules will have an average orientation perpendicular to the field and give rise to a sharp doublet with one-half of the original splitting. Initial orientation with the normal perpendicular to the field would actually produce a broad powder pattern after rotation. The former behavior was observed and an initial parallel orientation deduced.

From the data presented in this paper, particularly the ^{31}P data, CHAPSO-DMPC disks are oriented with bilayer normals orthogonal to the field. The single, oriented ^{31}P resonance grows from the 90° edge of the powder pattern of the random dispersion. This experiment has been repeated with the bile salt-DMPC system, and the 90° orientation of the bilayer normal is also observed. The discrepancy can be explained if exchange between HDO in the isotropic aqueous phase and associated with the interface is allowed. Both initial orientations then give rise to a pair of sharp lines with a factor of 2 reduction after a 90° rotation. Thus, no conclusions about orientation should have been based on the HDO splitting data.

CONCLUSIONS

The field-induced orientational behavior of the CHAPSO-DMPC system has been characterized, hopefully laying the ground work for its use in biochemically relevant membrane studies. The CHAPSO system offers the general advantage of sensitivity and resolution but also provides a very reproducible and rapid means of producing oriented samples. An additional advantage lies in the orientational toleration exhibited by at least certain samples to large variations of temperature, pH, and ionic strength as well as the addition of significant amounts of other amphiphiles. Thus, its application to a wide range of NMR biochemical studies would appear to be promising. Some perturbation of surface anchored molecules such as dodecyl melibioside by the addition CHAPSO can be

demonstrated, but it may be possible to produce other neutral or zwitterionic bile acid derivatives which allow the basic properties of CHAPSO-DMPC interactions to be maintained while chemically editing out the perturbing influence of CHAPSO upon the nature of the DMPC-buffer interface.

The authors thank Peter Demou for his assistance in running NMR experiments. The work was supported by grants GM19035 and GM33225 from the United States Public Health Service. Support for C. R. Sanders was provided by a Public Health Service National Research Service Award GM13227-01 from the National Institute of General Medical Sciences.

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